09 Rec'd PCT/PTO 06 MAR 1998

FORM PTO-1390 (REV 10-97) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

1209-122P

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/029579 NEW

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/SE 96/01119	06 September 1996	08 September 1995

TITLE OF INVENTION: METHODS AND COMPOSITIONS FOR NUCLEIC ACID TARGETING

APPLICANT(S) FOR DO/EO/US: LANDEGREN, Ulf

Applicant herewith submits to the Untied States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau). WO 97/09069
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(3)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(2)).
   a. ☒ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been make and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information:

    International Search Report (PCT/ISA/210)
    International Preliminary Examination Report (PCT/IPEA/409)

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
NEW	PCT/SE 96/01119	1209-122P

17. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5):

Search Report has been prepared by the EPO or JPO **$930.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) **$720.00**

No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)). . . . **$790.00**

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$1,070.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4). **$98.00**

CALCULATIONS	PTO USE ONLY

ENTER APPROPRIATE BASIC FEE AMOUNT =	**$ 1,070.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	**$ 130.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	11 - 20 =	0	X **$22.00**	**$ 0.00**	
Independent Claims	2 - 3 =	0	X **$82.00**	**$ 0.00**	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)		Yes	+ **$270.00**	**$ 270.00**	
TOTAL OF ABOVE CALCULATIONS =				**$ 1,470.00**	
Reduction of ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				**$ 0.00**	
SUBTOTAL =				**$ 1,470.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				**$ 0.00**	
TOTAL NATIONAL FEE =				**$ 1,470.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				**$ 0.00**	
TOTAL FEES ENCLOSED =				**$ 1,470.00**	
				Amount to be: refunded	**$**
				charged	**$**

a. ☒ A check in the amount of $ **1,470.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account. No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-2448. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

Send all correspondence to:
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(703)205-8000

SIGNATURE

MURPHY, GERALD M., JR.
NAME

28,977
REGISTRATION NUMBER

/gfo 03/06/98

Applicant or Patentee: Ulf LANDEGREN

Attorney's Docket No.: 1209-122P

Serial or Patent No.: 09/029,579

Filed or Issued: March 6, 1998

For: METHODS AND COMPOSITIONS FOR NUCLEIC ACID TARGETING

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27 (b)) — INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled METHODS AND COMPOSITIONS FOR NUCLEIC ACID TARGETING described in:

( ) the specification filed herewith
(X) application serial no. _____, filed March 6, 1998
( ) patent no. _____, issued _____.

I have not assigned, granted, conveyed, or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

(X) no such person, concern, or organization
( ) persons, concerns or organizations listed below*

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

FULL NAME _____

ADDRESS _____

( ) INDIVIDUAL ( ) SMALL BUSINESS CONCERN ( ) NONPROFIT ORGANIZATION

FULL NAME _____

ADDRESS _____

( ) INDIVIDUAL ( ) SMALL BUSINESS CONCERN ( ) NONPROFIT ORGANIZATION

FULL NAME _____

ADDRESS _____

( ) INDIVIDUAL ( ) SMALL BUSINESS CONCERN ( ) NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF INVENTOR	NAME OF INVENTOR	NAME OF INVENTOR
Ulf LANDEGREN		
Signature of Inventor	Signature of Inventor	Signature of Inventor
X		
Date	Date	Date
X APRIL 21, 1998		

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09/029579

PATENT
1209-0122P

IN THE U.S. PATENT AND TRADEMARK OFFICE

APPLICANT: LANDEGREN, Ulf

INT'L. APPLN. NO.: PCT/SE 96/01119

SERIAL NO.: GROUP:

FILED: March 6, 1998 EXAMINER:

FOR: METHODS AND COMPOSITIONS FOR NUCLEIC ACID TARGETING

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Box Patent Applications
Washington, D.C. 20231

March 6, 1998

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

IN THE SPECIFICATION:

Before line 1, insert --This application is the national phase under 35 U.S.C. §371 of prior PCT International Application No. PCT/SE 96/01119 which has an International filing date of September 6, 1996 which designated the United States of America, the entire contents of which are hereby incorporated by reference.--

R E M A R K S

The specification has been amended to provide a cross-reference to the previously filed International Application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §1.16 or under 37 C.F.R. §1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____ #34,330
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GMM/gfo

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PCT/SE96/01119
09/029579

METHODS AND COMPOSITIONS FOR NUCLEIC ACID TARGETING

Field of the invention

The present invention relates to methods and pharmaceutical compositions for targeting nucleic acid sequences, more specifically double stranded nucleic acid sequences. The compositions comprise oligonucleotides in the form of so called padlock probes. The padlock probes have two free nucleic acid end parts which are at least partially complementary to and capable of hybridizing with two at least substantially neighboring respective regions of a target nucleic acid sequence. Furthermore, the invention relates to use of said compositions as medicaments for treating genetic disorders.

Background of the invention

Oligonucleotides as potential therapeutics has developed by the ability to synthesize oligonucleotides, chemically modified oligonucleotide analogs and conjugated oligonucleotides, of suitable quantity and purity, as a result of the now ready availability of oligonucleotides through automated synthesis using, for example, the phosphoramidite method.

A first approach to therapeutic use of oligonucleotides is to use them as inhibitors of translation, with the complementary or 'antisense' base sequence targeted to a specific 'sense' sequence in the mRNA. In this way, expression of a specific protein can be regulated or inhibited.

Mechanisms of antisense inhibition include interference with ribosome binding and processing of mRNA conformation or mRNA splicing, and RNAase-H activation of mRNA digestion. The preferred target for antisense inhibition is the 5'-initiation codon.

A second approach to therapeutic use of oligonucleotides is to target DNA therewith and thereby directly inhibit gene function by inhibiting transcription to mRNA. In contrast to mRNA which,

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although extensively folded, is readily accessible, the DNA duplex is very stable which complicates inhibition thereof.

One way of solving the problem with inaccessibility of double stranded DNA is to take advantage of the fact that a third strand can be accommodated in the major groove of the B-form DNA duplex to form a triplex structure.

Duplex recognition by an oligonucleotide involves the formation of two hydrogen bonds with the purines of Watson-Crick base pairs within the major groove of the double helix. Thymine, cytosine, and guanine can adopt two different orientations called 'Hoogsteen' and 'reverse Hoogsteen' by analogy with the hydrogen-bonding scheme discovered by Hoogsteen in co-crystals of A and T derivatives. In contrast, adenine and inosine can form two hydrogen bonds with and A.T base pair in a single orientation. It should be noted that in order to form two hydrogen bonds with G, cytosine must be protonated. Therefore, triplets involving C+ x G.C are more stable at acidic pH. Methylation at C-5 of cytosine also contributes to stabilization of the triple helix.

Several mechanisms exist by which triple helix formation can alter gene transcription:

1. Triple helix formation within the promoter region can change DNA conformation and therefore alter the rate and efficiency of RNA polymerase initiation. This can lead to either activation or inhibition of transcription.
2. Oligonucleotide binding to a DNA sequence overlapping a transcription factor binding site may inhibit its transactivating capacity.
3. Triplex formation within or adjacent to the region where RNA polymerase binds may inhibit transcription initiation even if RNA polymerase and transcription factors are still bound to the promoter.
4. Oligonucleotide binding downstream of the RNA polymerase recognition site might inhibit progression of the transcription machinery along the DNA and therefore block RNA elongation.

Targeting by triple helix formation is limited to only a particular subset of DNA sequences, such as those associated with homopurine-homopyrimidine tracts.

An alternative way of directly inhibiting DNA is described in Nucleic Acids Research, 1993, Vol 21, No 2, p 197-200 to Nielsen et al. The authors describe that PNA (peptide nucleic acids chimera), i.e., DNA analogues in which the deoxyribose phosphate backbone has been replaced with a peptide backbone consisting of (2-amoniethyl)glycine units have retained the hybridization properties of DNA. There is shown that PNA binds more strongly to complementary oligonucleotides than DNA itself. Moreover, PNA can bind sequence specifically to double stranded DNA. This binding takes place by strand displacement rather than by triple helix formation. In brief, a rather unstable strand displacement complex is first formed with only one PNA molecule bound to the target by Watson-Crick hydrogen bonding, and this is subsequently trapped by binding of a second PNA molecule via Hoogsteen hydrogen bonding.

However, because of their relatively strong binding the sequence specificity rapidly diminishes with the increasing length of the PNA probes.

Branch capture reactions (BCRs) target duplex restriction fragments terminating in overhanging bases with short homologous single stranded DNA oligonucleotides that can pair with the unpaired overhanging bases and some flanking sequence so that complete base pairing displaces the end of one resident strand by branch migration. The limitation of BCRs is that they are limited to targeting only known terminal sequences and are, thus, not very suitable as therapeutic agents.

In Nature Genetics, vol. 3, april 1993, there is described another probe-targeting method which uses Rec A protein-coated short single stranded DNA probes to form four stranded hybrids between probes and duplex DNA targets. With this method in-

ternally localized sites can be targeted and the four stranded hybrids are stable.

All the above nucleic acid targeting methods suffer from drawbacks the most important one being the insufficient sequence specificity of the probes. This is an especially essential consideration in respect of the potential use of the probes as therapeutics.

Summary of the invention

The present invention is derived from the copending international application no. PCT/SE95/00163 entitled: Method, reagent and kit for detection of specific nucleotide sequences. This application is referred to and herein incorporated by reference. In this application so called padlock probes are described.

In summary, said application describes a probe designed to be circularized in the presence of a target sequence, wherein said probe is caused to close around the target nucleic acid, for example DNA or RNA, such that the cyclic probe will interlock with and thereby be efficiently linked to the target nucleic acid in a manner similar to "padlocks". The circularization of the probe ends is achieved with, for example, ligase. Such covalent catenation of probe molecules to target sequences result in the formation of an extremely stable hybrid.

It has now been surprisingly found that these padlock probes are able to affect gene function directly by binding to double stranded nucleic acids, without a prior denaturation step, and thereby affect the replication and transcription of the bound molecule. This is expected to provide new therapeutic possibilities for in vivo manipulation of gene sequences and treatment of genetic disorders.

In a first aspect, the present invention provides a method for targeting double stranded nucleic acids, comprising the following steps:

a) contacting a linear padlock probe
having two free nucleic acid end parts which are at least partially complementary to and capable of hybridizing with two at least substantially neighboring respective regions of a target nucleic acid sequence;

with a double stranded nucleic acid target without prior denaturation of said target;

b) hybridizing said free nucleic acid end parts with said two at least substantially neighboring respective regions of a target nucleic acid sequence; and
c) circularization of said padlock probe by joining said free end parts.

The joining in step c) is performed with a linking agent such as a ligase enzyme or mutually chemically reactive compounds at the free end parts.

The method of the invention can be performed both in vitro and in vivo.

According to a second aspect, the present invention provides a pharmaceutical composition for targeting double stranded nucleic acids, comprising an effective amount of a padlock probe oligonucleotide having two free nucleic acid end parts which are at least partially complementary to and capable of hybridizing with two at least substantially neighboring respective regions of a target nucleic acid sequence so that the padlock probe can be circularized by joining said free end parts and catenate with the target sequence for direct inhibition thereof.

The composition is preferably formulated in admixture with a suitable carrier, such as conventional pharmaceutically acceptable carriers known in the art.

According to a third aspect of the invention the above described

compositions are used as a medicament for treating genetic disorders.

Detailed description of the invention

Padlock probe targeting to double stranded DNA according to the method of the invention optionally involves a linking agent which can be chemical or biological. It is, for example, a ligase-assisted reaction. The principle employed in such a reaction is that a linear two-probe segment with a probe in each end, complementary to two target sequences situated in juxtaposition, are joined to a contiguous circular probe sequence with the aid of a linking agent, such as a DNA ligase. Examples of ligases are T4 DNA ligase, T7 DNA ligase, E.coli DNA ligase, and Thermus thermophilus DNA ligase. Also groups that are mutually chemically reactive may be used to join the ends of the probes in an enzyme-independent manner. This way of joining oligonucleotide ends has been previously used in the art. Besides ligases, proteins like RecA or single strand-binding protein can enhance the ability of circularizable probes to hybridize and become catenated to, base paired DNA.

The compositions according to the invention may or may not contain a linking agent depending on the use of the compositions. In vivo, RecA and DNA ligase are already present, and thus the addition of a linking agent may not be necessary for therapeutic applications.

According to the present invention, padlock probes are used in in vitro methods to specifically detect DNA sequences within a cell, without a requirement for prior denaturation. In this manner, for example, the correct spatial relations between specific DNA sequences can be analyzed without artificially induced effects.

In the in vitro method of the invention, probes of this type could also be used to modify and thereby mutate specific genes in in vitro cell lines, and for instance in embryonal stem cells

to give rise to transgenic animals carrying mutations in predefined genes.

In all these various applications, the effects of the padlock probes may be accentuated by at least partially building the probes of non-natural nucleic acids, or of polymers such as PNA, having advantages such as stronger base pairing, greater resistance to nucleases, or increased ability to cross cell membranes.

Padlock probes bind selectively and stably to double stranded DNA and enable sequence specific modification of DNA. In fact, it is contemplated that padlock probes even will be able to selectively bind gene sequence variants with point mutations, in order to inhibit the expression of the mutant genes, since the ligation is dependent upon the exact target sequence. The increased specificity is achieved by the fact that two shorter probe segments have to cooperate for binding to occur. A further advantage is that padlock probes are not sensitive to exonucleases due to their circular shape when they are ligated. On the other hand, excess of padlock probes is rapidly degraded by exonucleases which is a benefit in, for example, drug formulation.

The invention will now be illustrated further, by way of example only, by the following non-limiting specific Examples.

EXAMPLE 1

Padlock probe binding to double stranded nucleic acid target

A padlock probe oligonucleotide having the following sequence: 5' P-TGG TGT TTC CTA TGA-$((HEG_2)C\text{-}B)_4(HEG)_2$-AAG AAA TAT CAT CTT-3', wherein P is a phosphate residue, HEG is hexaethylene glycol and C-B is a biotinylated C residue, was synthesized using a commercial DNA synthesizer. The two ends of the oligonucleotide were capable of base-pairing adjacent to each other with exon 9 of the CTFR gene contained in the double stranded plasmid pUC 19.

The probe was labeled by exchanging the present 5' phosphate residue with ^{32}P using polynucleotide kinase and was allowed to hybridize with the target sequence. In a volume of 20µl 2 pmole probe were mixed with 0.2 pmole of plasmid in the presence or absence of 24 pmole RecA protein in a solution of 10mM Tris, pH 7.5, 10 mM $Mg(Ac)_2$, 50 mM KAc, 2 mM ATP with 5 units T4 DNA ligase and was incubated for 30 minutes at 37°C.

After incubation, washing was performed under non-hybridizing conditions. Thereafter, the reaction products were separated on a denaturing 6% polyacrylamide gel and the radioactive label was quantified in a Phosphorimager (Molecular Dynamics). The results clearly showed comigration, demonstrating invasion and binding of the above padlock probe to the double stranded plasmid, both in the presence and absence of RecA.

EXAMPLE 2

Padlock probe binding to double stranded nucleic acid target and inhibition of promotor

A 90-mer padlock probe with two 20 nucleotide end regions, capable of hybridizing in juxtaposition on one strand of the insert cloned in a Bluescript plasmid, was allowed to hybridize to a denatured, amplified fragment of the insert, and including the two transcriptional promoters T3 and T7, flanking the insert. One ng of amplification product was mixed with 20 pmol of padlock probe in a 10µl reaction with 10U of *Tth* ligase (Epicenter Technologies) in the presence of a NAD+-containing buffer, as recommended by the manufacturer. This buffer was previously shown to be well suited also for transcription by both the T3 and T7 RNA polymerases. The presence of a padlock probe on the double stranded amplified fragment efficiently interferred with transcription of both strands of the amplified fragment.

AMENDED CLAIMS

[received by the International Bureau on 17 January 1997 (17.01.97); original claims 1 - 13 replaced by amended claims 1 - 7 (1 page)]

1. A pharmaceutical composition for targeting double stranded nucleic acids, **characterized in** that it comprises an effective amount of a padlock probe oligonucleotide having two free nucleic acid end parts which are at least partially complementary to and capable of hybridizing with two at least substantially neighboring respective regions of a target nucleic acid sequence so that the padlock probe can be circularized by joining said free end parts and catenate with the target sequence for direct inhibition thereof.

2. A composition according to claim 1, in admixture with a suitable carrier.

3. A composition according to claim 1, also comprising a linking agent.

4. A composition according to claim 3, **wherein** linking agent is a ligase enzyme.

5. A composition according to claim 1, comprising mutually chemically reactive compounds at said end parts.

6. A composition according to any of claims 1-5, **wherein** said padlock probe comprises non-natural nucleic acids or polymers.

7. A composition for targeting nucleic acids, comprising an effective amount of a padlock probe having two free nucleic acid end parts which are at least partially complementary to and capable of hybridizing with two at least substantially neighboring respective regions of a target nucleic acid sequence so that it can be circularized and catenate with the target sequence, for use as a medicament.

BIRCH, STEWART, KOLASCH & BIRCH, LLP

PLEASE NOTE: YOU MUST COMPLETE THE FOLLOWING:

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT AND DESIGN APPLICATIONS

ATTORNEY DOCKET NO. 1209-122P

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated next to my name; that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Insert Title: METHODS AND COMPOSITIONS FOR NUCLEIC ACID TARGETING

Fill in Appropriate Information - For Use Without Specification Attached:

the specification of which is attached hereto. If not attached hereto,

the specification was filed on March 6, 1998 as United States Application Number _____; and /or

the specification was filed on September 6, 1996 as PCT International Application Number PCT/SE 96/01119; and was amended under PCT Article 19 on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I do not know and do not believe the same was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months (six months for designs) prior to this application, and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns, except as follows.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Insert Priority Information: (if appropriate)

Prior Foreign Application(s) (Number)	(Country)	(Month/Day/Year Filed)	Priority Claimed
9503117-5	Sweden	09/08/95	☒ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

Insert Provisional Application(s): (if any)

(Application Number) (Filing Date)

(Application Number) (Filing Date)

All Foreign Applications, if any, for any Patent or Inventor's Certificate Filed More Than 12 Months (6 Months for Designs) Prior To The Filing Date of This Application:

Insert Requested Information: (if appropriate)

Country	Application No.	Date of Filing (Month/Day/Year)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Insert Prior U.S. Application(s): (if any)

(Application Number) (Filing Date) (Status - patented, pending, abandoned)

(Application Number) (Filing Date) (Status - patented, pending, abandoned)

I hereby appoint the following attorneys to prosecute this application and/or an international application based on this application and to transact all business in the Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the attorneys identified below, unless the inventor(s) or assignee provides said attorneys with a written notice to the contrary:

Terrell C. Birch	(Reg. No. 19,382)	Raymond C. Stewart	(Reg. No. 21,066)
Joseph A. Kolasch	(Reg. No. 22,463)	James M. Slattery	(Reg. No. 28,380)
Bernard L. Sweeney	(Reg. No. 24,448)	Michael K. Mutter	(Reg. No. 29,680)
Charles Gorenstein	(Reg. No. 29,271)	Gerald M. Murphy, Jr.	(Reg. No. 28,977)
Leonard R. Svensson	(Reg. No. 30,330)	Terry L. Clark	(Reg. No. 32,644)
Andrew D. Meikle	(Reg. No. 32,868)	Marc S. Weiner	(Reg. No. 32,181)
Joe McKinney Muncy	(Reg. No. 32,334)	Andrew F. Reish	(Reg. No. 33,443)
C. Joseph Faraci	(Reg. No. 32,350)	Donald J. Daley	(Reg. No. 34,313)

16

Send Correspondence to: **BIRCH, STEWART, KOLASCH & BIRCH, LLP**

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PLEASE NOTE: YOU MUST COMPLETE THE FOLLOWING:

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

Full Name of First or Sole Inventor: Insert Name of Inventor Insert Date This Document is Signed

GIVEN NAME	FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Ulf	LANDEGREN		APRIL 21, 1998

Insert Residence Insert Citizenship

Residence (City, State & Country)	CITIZENSHIP
Uppsala, SWEDEN SE	SWEDEN

Insert Post Office Address

POST OFFICE ADDRESS (Complete Street Address including City, State & Country)

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Full Name of Second Inventor, if any:

see above

GIVEN NAME	FAMILY NAME	INVENTOR'S SIGNATURE	DATE*

Residence (City, State & Country)	CITIZENSHIP

POST OFFICE ADDRESS (Complete Street Address including City, State & Country)

Full Name of Third Inventor, if any

see above

GIVEN NAME	FAMILY NAME	INVENTOR'S SIGNATURE	DATE*

Residence (City, State & Country)	CITIZENSHIP

POST OFFICE ADDRESS (Complete Street Address including City, State & Country)

Full Name of Fourth Inventor, if any

see above

GIVEN NAME	FAMILY NAME	INVENTOR'S SIGNATURE	DATE*

Residence (City, State & Country)	CITIZENSHIP

POST OFFICE ADDRESS (Complete Street Address including City, State & Country)

Full Name of Fifth Inventor, if any

see above

GIVEN NAME	FAMILY NAME	INVENTOR'S SIGNATURE	DATE*

Residence (City, State & Country)	CITIZENSHIP

POST OFFICE ADDRESS (Complete Street Address including City, State & Country)

SCANNED